Health Council of the Netherlands

Perfluorooctanoic acid and its salts

Evaluation of the carcinogenicity and genotoxicity

2013/32

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : aanbieding advies *Perfluorooctanoic acid and its salts*
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Graag bied ik u hierbij het advies aan over de gevolgen van beroepsmatige blootstelling aan perfluoroctaanzuur en zijn zouten.

Dit advies maakt deel uit van een uitgebreide reeks waarin kankerverwekkende stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste subcommissie van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS), de Subcommissie Classificatie van carcinogene stoffen. Het advies is getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Perfluorooctanoic acid and its salts

Evaluation of the carcinogenicity and genotoxicity

Subcommittee on the Classification of Carcinogenic Substances of the
Dutch Expert Committee on Occupational Safety,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2013/32, The Hague, 18 december 2013

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research..." (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

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Samenvatting

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid evalueert en beoordeelt de Gezondheidsraad de kankerverwekkende eigenschappen van stoffen waaraan mensen tijdens de beroepsmatige uitoefening kunnen worden blootgesteld. In het voorliggende advies neemt de Subcommissie Classificatie van carcinogene stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen (GBBS) van de Raad, die deze evaluatie en beoordeling verricht, perfluoroctaanzuur en zijn zoutvormen onder de loep. Perfluoroctaanzuur en zijn zouten zijn moeilijk afbreekbare stoffen die onder andere gebruikt worden voor de vorming van fluoropolymeren die gebruikt worden in de antiaanbaklagen van pannen en het water- en vuilafstotend maken van materialen.

Op basis van de beschikbare gegevens meent de commissie dat deze niet voldoende zijn om de kankerverwekkende eigenschappen van perfluoroctaanzuur en zijn zouten te beoordelen (categorie 3).*

* Volgens het classificatiesysteem van de Gezondheidsraad (zie bijlage F).

Executive summary

At request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates and judges the carcinogenic properties of substances to which workers are occupationally exposed. The evaluation is performed by the Subcommittee on Classication of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council (DECOS). In this report, the Committee evaluated perflourooctanoic acid (PFOA) and its salts. PFOA and its salts are persistent compounds that are amongst others used to manufacture stick-resistant cookware and stain- and water-resistant fabrics.

The Committee is of the opinion that the available data on perfluorooctanoic acid and its salts are insufficient to evaluate the carcinogenic properties (category 3).*

* According to the classification system of the Health Council (see Annex F).

Chapter 1

Scope

1.1 Background

In the Netherlands a special policy is in force with respect to occupational use and exposure to carcinogenic substances. Regarding this policy, the Minister of Social Affairs and Employment has asked the Health Council of the Netherlands to evaluate the carcinogenic properties of substances, and to propose a classification (see Annex A). In addition to classifying substances, the Health Council also assesses the genotoxic properties of the substance in question. The assessment and the proposal for a classification are expressed in the form of standard sentences (see Annex F).

This report contains the evaluation of the carcinogenicity of perfluorooctanoic acid and its salts.

1.2 Committee and procedures

The evaluation is performed by the Subcommittee on Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter the Committee. The members of the Committee are listed in Annex B. The submission letter (in English) to the Minister can be found in Annex C.

In June 2013 the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft are listed in Annex D. The Committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The evaluation and recommendation of the Committee is based on scientific data, which are publicly available. The starting points of the Committees' reports are, if possible, the monographs of the International Agency for Research on Cancer (IARC). This means that the original sources of the studies, which are mentioned in the IARC-monograph, are reviewed only by the Committee when these are considered most relevant in assessing the carcinogenicity and genotoxicity of the substance in question. In the case of perfluorooctanoic acid and its salts, no IARC monographs are available.

More recently published data were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last updated online search was in November 2013. The recovered relevant data were included in this report.

Chapter 2

General information

2.1 Introduction

The data have been retrieved from the European Substance Information System (ESIS)* and the Hazardous Substances Data Bank (HSDB**). Perfluorooctanoic Acid (PFOA) is a fully fluorinated carboxylic acid. PFOA is a strong acid and dissociates in biological media. PFOA is mainly used and produced as its highly soluble ammonium salt (APFO, ammoniumperfluorooctanoate). There are other perfluorooctanoate salts as well, i.e. sodium, potassium and silver salts. Relevant physico-chemical properties of PFOA are presented below.

Chemical name	:	Perfluorooctanoic acid
CAS registry number	:	335-67-1
EINECS number	:	206-397-9
Synonyms	:	Pentadecafluorooctanoic acid Perfluorocaprylic acid Perfluoroctanoic acid Perfluoroheptanecarboxylic acid Perfluorooctanoic acid
Appearance	:	Solid

* ESIS can be accessed via the ECB-site: http://esis.jrc.ec.europa.eu/ (accessed October 8, 2013).

** HSDB can be accessed via: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (accessed October 8, 2013).

Use	:	Perfluorooctanoic acid is used primarily to produce its salts, which are used as essential processing aids in the production of fluoropolymers and fluoroelastomers.
Chemical formula	:	C8HF15O2
Molecular weight	:	414.09 g/mol
Boiling point	:	189˚C
Melting point	:	52-54˚C
Vapour pressure	:	0.15 mm Hg at 25˚C /
Vapour density (air=1)	:	-
Solubility	:	In water: 9500 mg/L
Conversion factor	:	-
EU Classification (100% solution)	:	Not classified

2.2 IARC classification

Currently PFOA has not been evaluated by IARC, but it is mentioned on the list of priority agents for future IARC Monographs.

Chapter 3

Carcinogenicity studies

Epidemiological and experimental studies on PFOA and its ammonium salt APFO were recently reviewed by Steenland et al. (2010)[1] and Post et al. (2012)[2]. The Committee consulted the ECHA-RAC reports on PFOA and APFO (2011)[3,4], and the evaluations of the C8 Health Project and the C8 Science Panel (2012)[5].

3.1 Observations in humans

Human studies concerning cancer are mainly restricted to two US occupational populations (employees from 3M (Gilliland and Mandel, 1993[6]; Alexander, 2001[7]; Lundin et al., 2009[8]) and DuPont (Leonard et al., 2008[9]; Steenland and Woskie, 2012[10]). One occupational study by Consonni et al. (2012)[11] included employees in production sites in both Europe and US. In addition, two studies on cancer in residents living in the vicinity of a DuPont plant were published (Vieira et al., 2013[12]; Barry et al., 2013[13]). Three studies were published on the general population in Denmark (Eriksen et al., 2009[14]), Greenland (Bonefeld-Jorgensen et al., 2011[15]) and Sweden (Hardell et al., 2013[16]).

3.1.1 Occupationally exposed employees

Gilliland and Mandel[6] examined the relationship between ammonium perfluorooctanoate (APFO) and mortality using a retrospective cohort mortality design.

This study was performed on employees at the 3M Cottage Grove, Minnesota plant which produces APFO. At this plant, APFO production was limited to the Chemical Division. The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. Death certificates of all of the workers were obtained to determine cause of death. There was almost complete follow-up (99.5%) of all of the study participants. The exposure status of the workers was categorized based on their job histories. If they had been employed for at least 1 month in the Chemical Division, they were considered exposed. All others were considered to be not exposed to PFOA. The number of months employed in the Chemical Division provided the cumulative exposure measurements. Of the 3,537 (2,788 men and 749 women) employees who participated in this study, 398 (348 men and 50 women) were deceased. Eleven of the 50 deceased women and 148 of the 348 deceased men worked in the Chemical Division, and therefore, were considered exposed to PFOA.

The observed numbers of cause-specific deaths were compared to the expected numbers of deaths obtained by applying sex- and race-specific quinquennial age, calendar period, and cause-specific mortality rates for the United States and Minnesota populations to the distribution of observed person-time. Because less than 1% of plant employees were non-white, white male and white female rates were used for comparison. For women, only United States rates were used because cause- and calendar period-specific Minnesota rates for women were not available. The effects of latency, duration of employment and work in the Chemical Division were examined using stratified standardized mortality ratio (SMR) analyses. SMRs for males were calculated for three latency intervals (10, 15, and 20 years) and three categories of duration of employment (5, 10, and 20 years).

In women, the SMR for all causes of death (SMR=0.75; 95% CI=0.56-0.99) was significantly lower than expected. The SMR for all cancers in women (both exposed and not exposed) was 0.71 (95% CI=0.42-1.14). There was no association with duration of employment or latency for deaths from all causes of death or cancer. Mortality among Chemical Division women was significantly less than expected. In Chemical Division women, the all-causes SMR was 0.46 (95% CI=0.23-0.86) and the cancer SMR was 0.36 (95% CI=0.07-1.05). The all-causes SMR for the non-Chemical Division women was 0.91 (95% CI=0.64-1.24) and the cancer SMR was 0.91 (95% CI=0.49-1.52).

The only elevated (although not significant) SMR in women (both exposed and unexposed) was for lymphopoietic cancer (1.47; 95% CI=0.30-4.29), and was based on only 3 deaths.

Using Minnesota rates for comparison, the SMR for men for all causes of death was significantly less than 1 (0.77; 95% CI=0.69-0.86). In the Chemical Division, the all-causes SMR was 0.86 (95% CI=0.72-1.01); in the non-Chemical Division, the all-causes SMR was 0.69 (95% CI=0.59-0.79). The SMR for all cancers in men (both exposed and not exposed) was 1.05 (95% CI 0.86-1.27) using Minnesota rates for comparison; similar results were obtained when the expected number of male deaths was based on US mortality rates.

When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI=0.55-4.59). This was based on 4 deaths (1.97 expected). In the non-Chemical Division group, the SMR for prostate cancer was 0.58 (95% CI=0.07-2.09). There was a statistically significant (p=0.03) association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk of prostate cancer for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI=1.01-1.27). Ten years of employment in the Chemical Division was associated with an estimated 3.3-fold increase (95% CI=1.02-10.60) in prostate cancer mortality.

According to the Committee the study has several limitations. The low SMRs observed in the study are most likely a result of the healthy worker effect. The rates were based on small numbers of cases and produced unstable ratios. Estimates of APFO exposure were based on job history, and categorization of workers into ever versus never employed in the Chemical Division may not reflect the biologic effective dose of PFOA. The authors stated that APFO exposure was apparently widespread among employees not directly exposed to APFO; thus, this categorization might have also misclassified the workers as unexposed when they were exposed. Furthermore, workers were exposed to many other carcinogenic substances, such as benzene and asbestos, during their employment at the plant.

An update of the study of Gilliland and Mandel was conducted by Alexander (2001).[7,17] The update was conducted to include the death experience of employees through 1997. The cohort consisted of 3,992 workers. The eligibility requirement was increased to 1 year of employment at the Cottage Grove plant, and the exposure categories were changed to be more specific. Workers were placed into 3 exposure groups based on job history information: definite PFOA

exposure (n=492, jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (n=1,685, other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (n=1,815, primarily non-chemical division jobs).

In this new cohort, 607 deaths were identified: 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at p=0.05. The highest SMR reported was for bladder cancer (SMR=1.31, 95% CI=0.42-3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR=1.67, 95% CI=0.02-6.02), 1 from pancreatic cancer (SMR=1.34, 95% CI=0.03-7.42), and 1 from prostate cancer (SMR=1.30, 95% CI=0.03-7.20). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to experience cerebrovascular disease mortality (5 deaths observed, 1.94 expected; 95% CI=0.84-6.03).

In the probable exposure group, 3 SMRs were elevated: cancer of the testis and other male genital organs (SMR=2.75, 95% CI=0.07-15.3); pancreatic cancer (SMR=1.24, 95% CI=0.45-2.70); and malignant melanoma of the skin (SMR=1.42, 95% CI=0.17-5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 (95% CI=0.28-2.02) (n=5).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI=0.58-5.40).

According to the Committee it is difficult to interpret the results of the prostate cancer deaths between the first study and the update because the exposure categories were modified in the update. Only 1 death was reported in the definite exposure group and 5 were observed in the probable exposure group. All of these deaths would have been placed in the chemical plant employee exposure group in the first study. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported. This is important because even 1 prostate cancer death in the definite PFOA exposure group resulted in an elevated SMR for the group. Therefore, if any of the employees' exposures were misclassified, the results of the analysis could be altered significantly.

Subsequently, Lundin and coworkers[8] published another update of a cohort of 3,993 employees employed at the same plant, with a follow-up until 31 December 2002. The updated study identified 807 descendents in the follow-up period, while the original study of Gilliland and Mandel[6] identified 398. The authors estimated SMRs compared with the general population, and used time-dependent Cox regression models to estimate the risks using an internal-cohort reference population. The same classification of jobs regarding work history into 3 general categories of APFO exposure was used as in the study of Alexander (2001)[7]: "definite occupational exposure", "probable occupational exposure" and "no or minimal exposure". The authors incorporated 2 approaches for characterizing APFO exposure in the analysis. The primary analysis was based on ever attaining a minimum time in jobs with probable or definite exposure. A secondary analysis was a cumulative exposure model with a weighted exposure based on duration of employment and qualitatively-specified exposure intensity.

The exposure weights derived were based partially on the serum APFO concentrations collected in 2000 from 131 employees in the chemical division of the plant. For workers that were classified as definite exposure, the plasma levels were ranging from 2.6 to 5.2 µg/ml, while for the probable exposed workers they were 0.3-1.5 µg/ml. No data were available for the jobs in the unexposed areas of the plant. The initial cumulative exposure assigned weights of 1 in jobs with no exposure, 30 in jobs with probable exposure and 100 in jobs with definite exposure. These weighting factors, although somewhat arbitrary, were chosen to reflect the relative exposure intensity of jobs and long biological half-life of APFO, implying that short-term peak exposures may equate to longer-term lower exposures over time. The cumulative exposure was calculated for each worker as a sum of the days of employment at each level, multiplied by the exposure weighting factor (weighting exposure level x number of days exposed). The cumulative exposure was categorized into groups selected *a priori*, representing an equivalent of up to 1 year (36,499 exposure-days), 1-4.9 years (36,500-182,499 exposure-days) and 5 or more years (182,500 exposure-days) of employment in a job with definite exposure.

To explore potentially confounding effects of the socioeconomic status between the workers, the authors further classified cohort members by wage type: hourly, salaried or both. The last was designated if the job history included earning each type of wage for at least 365 days.

The mortality experience of the cohort was initially compared with the mortality rates for the state of Minnesota. The all-cause and cause-specific SMRs were first computed for the full cohort and then for the exposure-specific categories and wage type. Subsequently, to model the risk as a function of APFO

exposure using an internal referent population of non-exposed workers, hazard ratios (HRs) and 95% CIs were estimated. The time covariate was from date of entry into the cohort until death or end of follow-up. The models were adjusted for sex and year of birth. Next to wage type age at entry into the cohort and smoking status were also examined as potential confounding covariates. To explore potential effects of latency, the exposure models were lagged by 10 years. Because smoking data were unavailable for many of the cohort members, a multiple-imputation model was constructed using those with smoking data to predict the smoking status of those without smoking data. The predictors used for the imputation process were sex, year of birth, year of first employment at the facility, age at entry into the cohort, and wage type.

The cohort was mostly male (80%), particularly in the "definite exposure" subgroup (92%). There was a higher prevalence of smoking in those who ever had a job with definite APFO exposure (65%) compared with non-exposed workers (47%). However, smoking data were available for 66% of the definite-exposure subgroup, whereas it was available for only 20% of the non-exposed. A majority of workers holding "definite exposure" jobs were hourly employees, while most non-exposed workers were salaried.

The all-cause and cause-specific SMRs were generally lower for the entire cohort and for exposure subgroups than for the general population of Minnesota. The SMR (95% CI) for all cancers in the ever definite exposure group was 0.9 (0.5-1.4), 0.9 (0.8-1.1) in the ever probable exposure group and 0.8 (0.6-1.0) in the non-exposed group. The SMR for cohort members ever employed in jobs with definite APFO exposure was elevated for prostate cancer, although confidence intervals were wide (2.1; 95% CI=0.4-6.1). In contrast the number of deaths from prostate cancer was lower (3) than expected among the never-exposed and probably exposed members of the cohort (SMRs 0.4; 95% CI=0.1-0.9 and 0.9; 95% CI=0.4-18, respectively). The SMRs for other types of cancer were as follows: for billiary passages and liver primary cancer not estimable (0 deaths, 95% CI=0.0-7.6), 0.7; 95% CI=0.1-2.6 and 0.3; 95% CI=0.0-1.8 in the definite, probable and no exposure groups, respectively; for pancreas cancer 0.9; 95% CI=0.0-4.7, 1.0; 95% CI=0.4-2.1 and 0.7; 95% CI=0.2-1.6 in the definite, probable and no exposure groups, respectively; for trachea, bronchus and lung cancer 1.2; 95% CI=0.5-2.3, 1.0; 95% CI=0.7-1.4 and 0.8; 95% CI=0.5-1.1 in the definite, probable and no exposure groups, respectively; and for bladder and other urinary organs cancer not estimable (0 deaths, 95% CI=0.0-9.6), 1.2; 95% CI=0.3-3.5 and 1.4; 95 % CI=0.4-3.7 in the definite, probable and no exposure groups, respectively.

The SMRs for salaried workers indicated a decreased risk of death for all cancers combined (SMR=0.7 (95% CI=0.6-0.8), respiratory cancers (0.6; 95% CI=0.4-0.9) and prostate cancer (0.5; 95% CI=0.2-1.2). The results were somewhat different for hourly employees: SMRs were 1.0; 95% CI=0.9-1.2 for all cancers combined; 1.2; 95% CI=0.9-1.6 for respiratory cancers and 0.9; 95% CI=0.4-1.6 for prostate cancer.

In the time-dependent Cox regression models, compared with an internal referent population of non-exposed workers, moderate or high exposures to APFO were positively associated with prostate cancer (hazard ratios (HR) (95% CI)=3.0 (0.9-9.7) and 6.6 (1.1-37.7), respectively). However, no positive association was evident when compared to the general population. There was no association between exposure and risk of pancreatic, testicular or bladder cancer. Including wage type and smoking habit in the models did not alter the results. Lagging exposures by 10 years made unremarkable differences in the hazard ratio estimates.

The study had several limitations. The population used in this study was relatively small. The authors concluded that while an internal referent population may provide a more valid comparison (assuming similar social and demographic determinants of disease), it should be taken into account that the SMRs for the exposed categories were modestly above unity, while the non-exposed members of the cohort were markedly below. The authors also stipulated that this difference of the non-exposed and other men in Minnesota with respect to baseline prostate cancer disease risk may be related, in part, to socioeconomic status. Wage status was the only available proxy for socioeconomic status and thus did not fully capture the complexities of socioeconomic status and its relation to health. Some exposure misclassification could not be excluded as work history records were used. The smoking data were sparse, and although methods were applied to impute the missing data, the validity of these imputations is not clear. The mean age at follow-up was 60 years, and the relatively small number of deaths limited the ability of the study to examine exposure responses.

Leonard et al. (2008)[9] investigated whether ischemic heart disease mortality was increased in a cohort of employees with work history at a US polymer manufacturing facility (DuPont Washington Works (WW), Parkersburg West Virginia). The secondary objective of this study was to examine mortality for a broad range of other mortality causes, including cancer outcomes.

The cohort comprised 6,027 men and women who had worked at the facility between 1948 and 2002; these years delimit the mortality follow-up period.

Standardized mortality ratios (SMR) were estimated to compare observed numbers of deaths to expected numbers derived from mortality rates for 3 reference populations: the US population, the West Virginia state population, and an 8-state regional employee population from the same company. The comparison with this regional DuPont reference population was expected to minimize the healthy worker effect.

Most SMR estimates based on US and state populations were below 100. Comparison to the employee population also resulted in many SMR estimates at or near a no-effect level. Relative to the regional worker population, a non-significant elevation for ischaemic heart disease mortality was observed (SMR=109; 95% CI=96-124). Mortality associated with diabetes was significantly increased compared to the regional worker population (SMR=197; 95% CI=123-298). A corresponding increase in the SMR for ischaemic heart disease and diabetes mortality was not detected for comparisons with the two general populations.

Despite limited statistical power to evaluate mortality rates for specific cancers due to small numbers of observed death, some elevated mortality risks did emerge. For kidney cancer mortality, comparisons with all 3 respective reference populations (US general, state of West Virginia, Dupont regional) showed increased, but nonsignificant, SMR estimates (SMR=152, 95% CI=78-265; SMR=151; 95% CI=78-264; SMR=181 (95% CI=94-361). For bladder cancer mortality, comparison with regional DuPont reference population showed increased but non-significant SMR estimate (SMR=130, 95% CI=52-269). No excesses for liver, pancreas, testicular, or breast cancer were found (based on small numbers of deaths (8, 11, 1, 2).

The investigators conclude that the results reported show little evidence of increased cause-specific mortality risks for workers at the plant. This study also demonstrates the utility of comparing occupational cohorts with a similar worker reference population in order to reduce bias associated with the healthy worker effect.

Steenland and Woskie (2012)[10] updated the abovementioned mortality analyses (Leonard et al., 2008[9]) of the same cohort at a DuPont chemical plant in West Virginia with a follow up through 2008, increasing the number of death from 806 to 1,084. The authors studied the mortality of 5,791 workers exposed to PFOA, using a newly developed job exposure matrix based for 1,308 workers from 1979-2004 (Kreckman et al., 2009[18]). The authors used 2 referent groups: other DuPont workers in the region and the US population.

In comparison with other DuPont workers, cause-specific mortality was elevated for mesothelioma (SMR=2.85; 95% CI=1.05-6.20), diabetes mellitus (SMR=1.90; 95% CI=1.35-2.61), and chronic renal disease (SMR=3.11; 95% CI=1.66-5.32). Significant positive exposure-response trends occurred for both malignant and nonmalignant renal disease (12 and 13 deaths, respectively). No exposure-response trend was seen for diabetes or ischaemic heart disease mortality.

In conclusion, the authors found evidence of positive exposure-response trends for malignant and nonmalignant renal disease. These results were limited by small numbers and restriction to mortality data.

[The authors argued that the mesothelioma development was caused by historical exposure to asbestos in the plant.]

Consonni et al. (2012)[11] explored the occupational cancer risk of tetrafluoroethylene (TFE) in humans in a retrospective cohort mortality study (1950-2008) that included all polytetrafluoroethylene (PFE) production sites in Europe and North America at the time it was initiated. Tetrafluoroethylene (TFE), a compound used for the production of fluorinated polymers including polytetrafluoroethylene, increases the incidence of liver and kidney cancers and leukemia in rats and mice. Because polymerization involves the use of ammonium perfluoro-octanoate (APFO), the mortality rate in relation to exposure to this potential confounder was also examined in this study.

A job exposure matrix (1950-2002) was developed for TFE and APFO (Sleeuwenhoek & Cherrie, 2012).[19] National reference rates were used to calculate standardized mortality ratios (SMRs) and 95% confidence intervals.

Among 4,773 workers ever exposed to TFE, a lower rate of death from most causes was found, as well as increased risks for cancer of the liver (SMR=1.27; 95% CI=0.55-2.51; 8 deaths) and kidney and urinary cancer combined (SMR=1.44; 95% CI=0.69-2.65; 10 deaths) and for leukemia (SMR=1.48; 95% CI=0.77-2.59; 12 deaths). A nonsignificant upward trend (p=0.24) by cumulative exposure to TFE was observed for liver cancer.

The Committee takes note of the author's conclusion that TFE and APFO exposures were highly correlated, and therefore their separate effects could not be disentangled.

3.1.2 Residents in the vicinity of a chemical plant

In a case-control study Vieira et al. (2013)[12] investigated the relationship between PFOA exposure and cancer among residents living near the DuPont

Teflon-manufacturing plant in Parkersburg, West Virginia (WV). The study area consisted of six districts with a contaminated public water supply and 13 adjacent counties. The analyses included incident cases of 18 cancers diagnosed from 1996 through 2005 in five Ohio (OH) counties and eight WV counties.

For analyses of each cancer outcome, controls comprised all other cancers in the study data set except kidney, pancreatic, testicular, and liver cancers, which have been associated with PFOA in animal or human studies. Logistic regression models were applied to individual-level data to calculate adjusted odds ratios (AORs) and confidence intervals (CIs).

For the combined analysis of OH and WV data, the exposure of interest was the resident's water district. Within OH, geocoded addresses were integrated with a PFOA exposure model to examine the relationship between cancer odds and categories of estimated PFOA serum. Serum levels of PFOA were estimated using combined environmental, exposure and pharmacokinetic models.

The final data set included 7,869 OH cases and 17,238 WV cases. There was a positive association between kidney cancer and the very high and high serum exposure categories (AOR=2.0, 95% CI=1.0-3.9, n=9 and AOR=2.0,=1.3-3.2, n=22, respectively) and a null association with the other exposure categories compared with the unexposed. The largest AOR was found for testicular cancer with the very high exposure category (AOR=2.8, 95% CI=0.8-9.2, n=6), but there was an inverse association with the lower exposure groups, and all estimates were imprecise because of small case numbers.

According to the authors the results suggest that higher PFOA serum levels may be associated with testicular, kidney, prostate, and ovarian cancers and non-Hodgkin lymphoma.

The Committee notes some critical shortcomings in the study by Vieira et al. First, no information on actual drinking water consumption is available. A subject living on an address modeled as high exposure, but who drinks no tap water in reality is not exposed, but is yet classified as exposed. Second, no historical data on address of the subjects was available. Instead it was assumed that the subject had lived on his/her known address for the past ten years. As a result of these two weaknesses actual exposure through consumption of drinking water may very well have been quite different from what was modeled. Next, no information on risk factors, other than smoking was obtained. The results therefore have not been corrected for other known risk factors, which may lead to incorrect interpretation of the findings. In this study exposure status was associated with lung cancer and Non Hodgkin's Lymphoma (NHL), but just reached statistical significance for NHL (p=0.05). An adjustment for multiple comparison issues would have probably made both associations not-statistically-

significant. Furthermore, the authors conclude that the study demonstrates that high serum levels of PFOA may be associated with an excess risk of testicular cancer, prostate cancer, ovarian cancer and NHL. However, only for NHL there seems to be an association with exposure. The other three cancer sites were not significantly increased in the analysis by exposure status. The associations reported are very weak and are not in agreement with the cancer types that were most likely the *a priori* effect parameters (pancreas and prostate cancer (Steenland 2010, p. 1103).[1]

Barry et al. (2013)[13] examined cancer incidence in mid-Ohio valley residents exposed to PFOA in drinking water due to chemical plant emissions.

The cohort consisted of adult community residents who resided in contaminated water districts or worked at a local DuPont chemical plant.

Most participated in a 2005/2006 baseline survey in which serum PFOA was measured. The cohort was interviewed in 2008-2011 to obtain further medical history. Retrospective yearly PFOA serum concentrations were estimated for each participant from 1952-2011. Self-reported cancers were validated through medical records and cancer registry review. The association between cancer and cumulative PFOA serum concentration was estimated using proportional hazards models.

Participants (n=32,254) reported 2,507 validated cancers (21 different cancer types). Estimated cumulative serum PFOA concentrations were positively associated with kidney and testicular cancer (hazard ratio (HR)=1.10, 95% CI=0.98-1.24 and HR=1.34, 95% CI=1.00-1.79, respectively, for 1-unit increases in ln-transformed serum PFOA). Categorical analyses also indicated positive trends with increasing exposures for both cancers (kidney cancer HRs for increasing exposure quartiles=1.0, 1.23, 1.48, and 1.58, linear trend test p=0.18; testicular cancer HRs=1.0, 1.04, 1.91, 3.17, linear trend test p=0.04).

The authors conclude that PFOA exposure was associated with kidney and testicular cancer in this population. The authors also indicate that, because this is largely a survivor cohort, findings must be interpreted with caution, especially for highly fatal cancers such as pancreatic and lung cancer.

According to the Committee one of the weaknesses of this study by Barry et al. is the representativity of the study population for the underlying general population and worker population. Subjects with a diagnosis of cancer in their history might have been more likely to participate in the study than subjects without such a history, since the latter might think their participation as not so relevant. Second, a part of the person-years of follow-up were enumerated in the period that the regional cancer registries were not yet in existence. Cancer

confirmation in that period was thus restricted to the available medical records, sometimes going back 50 years or more in time. The availability of medical records over such a long period may have introduced serious bias. Thirdly, the cohorts were compiled in 2005-2006, and the subjects had to survive up to that date to be eligible for the study. This survivor effect can have serious effects on the cancer incidence after 2005-2006, a period with relatively many person-years contributed in the older age groups. An analysis of the person-years generated after 2005-2006 would not have diminished this potential serious bias. Finally no comparisons with an unexposed population were made. Therefore it is not possible to assess how the exposed population as a whole in terms of cancer incidence compared to an external general population. The results of this study are not unambiguous. The authors conclude that kidney cancer and testicular cancer were associated with PFOA exposure. However, the tables on dose response analyses for kidney cancer and testicular cancer provide only limited support for this conclusion. This is especially the case when the about tenfold higher exposure in the workers is considered.

3.1.3 General population

Eriksen and coworkers[14] investigated the association between plasma levels of PFOA anion and cancer risk in the general population. From December 1, 1993, through May 31 1997, a total of 57,053 individuals who were aged 50-65 years, born in Denmark and had no previous cancer diagnosis were enrolled in a prospective cohort. Up to 12 years after enrollment in the cohort a number of 713, 332, 128, and 67 patients with prostate, bladder, pancreatic, and liver cancer, respectìvely, was diagnosed. Plasma samples were obtained at recruitment and analyzed for PFOA by high pressure liquid chromatography coupled with tandem mass spectroscopy.

Data on potential confounders were obtained from detailed questionnaires administered at enrollment. In total, 1,240 cancer patients were identified, 1,111 of whom were men and 129 were women. Mean plasma concentrations of PFOA were higher for men than for women (5%-95% percentiles of 6.8 (95% CI=3.1-14.0) ng/mL and 6.0 (95% CI=2.6-11.0) ng/mL, respectively). The mean plasma concentrations (5%-95% percentiles) in patients with prostate cancer were 6.9 (95% CI=3.1-14.1) ng/mL, with bladder cancer 6.5 (95% CI=2.7-13.4) ng/mL, with pancreatic cancer 6.7 (95% CI=3.0-12.8) ng/mL, and with liver cancer 5.4 (95% CI=2.5-13.7) ng/mL.

Crude IRRs were calculated for the three upper quartiles of PFOA compared with the lowest quartile (quartile 2 vs 1, quartile 3 vs 1, and quartile 4 vs 1). The

adjusted IRRs were corrected for the detected confounders. The quartile analysis indicated no differences in IRRs in relationship to plasma concentrations of PFOA. The adjusted IRRs for prostate cancer for the three upper quartiles of perfluorooctanoate compared with the lowest quartile (reference) were 1.09 (95% CI=0.78-1.53) for the 2nd, 0.94 (95% CI=0.67-1.32) for the 3rd and 1.18 (95% CI=0.84-1.65) for the 4th quartile.

For bladder cancer they were 0.71 (95% CI=0.46-1.07 for the 2nd, 0.92 (95% CI=0.61-1.39) for the 3rd and 0.81 (95% CI=0.53-1.24) for the 4th quartile.

For pancreatic cancer they were 0.88 (95% CI=0.49-1.57) for the 2nd, 1.33 (95% CI=0.74-2.38) for the 3rd and 1.55 (95% CI=0.85-2.80) for the 4th quartile; and for liver cancer 1.00 (95% CI=0.44-2.23) for the 2nd, 0.49 (95% CI=0.22-1.09) for the 3rd and 0.60 (95% CI=0.26-1.37) for the 4th quartile.

Crude and adjusted IRRs were similar. No linear trend in risk in relation to PFOA concentrations was observed for any cancers examined. Sex did not modify the associations between the plasma concentrations of PFOA and adjusted cancer risk.

It was concluded that plasma concentrations of PFOA among people in the general population were not associated with risk of prostate, bladder, pancreatic, or liver cancer.

It should, however, be noted that mean plasma concentration in this general population were significantly lower than for potentially exposed or exposed workers in the study of Lundin et al.[8] (ca. 7 ng/mL vs. 0.3-5.2 µg/mL).

Bonefeld-Jorgensen et al. (2011)[15] observed (according to the authors) an extraordinary increase in breast cancer in the Inuit population of Greenland and Canada although still lower than in Western populations. A study was performed aiming at the evaluation of the association between serum levels of persistent organic pollutants and perfluorinated compounds in Greenlandic Inuit breast cancer cases and their controls.

Thirty-one breast cancer cases and 115 controls were sampled during 2000-2003 from various Greenlandic districts. The serum levels of persistent organic pollutants, perfluorinated compounds and some metals were determined. The student t-test was used to compare the differences and the odds ratios were estimated by unconditional logistic regression models. A significant association between serum perfluorinated compounds levels and the risk of breast cancer was observed.

The authors conclude that the level of serum perfluorinated compounds might be risk factors in the development of breast cancer in Inuit.

The Committee notes that the study population is rather limited (n=31). Associations were analysed with a large number of blood parameters, increasing the chance of accidental findings. Moreover, Inuit with elevated serum levels could have another pattern of nutrition compared to Inuit with low serum levels, the latter possibly being the real risk factor.

Hardell et al. (2013)[16] conducted a case-control study in Sweden on prostate cancer to investigate the association with perfluorinated alkyl acids (PFAA). Blood samples taken from 201 patients with prostate cancer and 186 controls were analyzed for 5 PFAA compounds including PFOA.

The blood concentrations for PFOA did not differ statistically significantly between cases and controls. Overall no association between prostate cancer and PFOA concentration was found (OR=1.1; 95% CI=0.7-1.7), although secondary hypothesis generating analyses suggested that for prostate cancer with a hereditary component there was a positive relationship with PFAA compounds (for PFOA: OR=2.6; 95% CI=1.2-6.0) but not for *de novo* cases.

3.2 Summary of observations in humans

Six epidemiological studies in workers (on three cohorts) which the Committee considered relevant for relationship between PFOA/APFO exposure with cancer were assessed [6-11]. In addition, two epidemiological studies on cancer in residents in the vicinity of a chemical plant[12,13] and three studies in exposed community populations[14-16] were evaluated.

After having reviewed the epidemiological studies conducted, the Committee draws the following overall conclusions: The conducted studies are of a varying quality and several suffer from significant weaknesses. Several studies report elevated risks for certain types of cancer. Overall however, there is no cancer type that is consistently elevated in these studies. The cancer type of highest concern according to the Committee is kidney cancer. However, no excess of kidney cancer was observed in the 3M worker cohort, kidney cancer risk in the DuPont worker cohort was in the expected range with a small but statistically significant excess in the highest exposed quartile only, and the multinational cohort mortality study by Consonni et al. reported an overall non significant excess of kidney cancer mortality lacking a dose response trend or association with duration of exposure.

The reported results of a relatively substantial number of human longitudinal studies have such a high degree of inconsistency that the Committee classifies the human data as inadequate for firm conclusions about whether or not a cancer risk exists from exposure to PFOA in these studies.

3.3 Carcinogenicity studies in animals

The carcinogenic potential of PFOA has been investigated in two dietary carcinogenicity studies in rats by Sibinski et al., (1987)[17,20] and Biegel et al., (2001)[21]. In addition a study with monkeys (Butenhoff et al., 2002)[22] has been retrieved. A study investigating the role of PFOA as a liver tumour promoter in rats initiated with dimethylnitrosamine will also be discussed (Abdellatif et al.[23-25]).

3.3.1 Studies with rats

Sibinski et al. (1987)[17,20] investigated the chronic toxicity and carcinogenic potential of APFO in a dietary study in rats. Groups of 50 male and 50 female Sprague-Dawley (Crl:CD BR) rats were fed diets containing 0, 30 or 300 ppm APFO for two years. Groups of 15 additional rats per sex were fed 0 or 300 ppm APFO and evaluated at the one year interim sacrifice. In males the mean test article consumption was 1.3 and 14.2 mg/kg bw/day for the 30 and 300 ppm groups, respectively; in females, the mean test article consumption was 1.6 and 16.1 mg/kg bw/day for the 30 and 300 ppm groups, respectively. Histologic evaluations showed lesions in the liver, testes and ovary. In the liver, the increased incidence of lesions reached statistical significance only in the high-dose male group. At the 1 year interim sacrifice, diffuse hepatomegalocytosis (12/15 animals), portal mononuclear cell infiltration (13/15 animals) and hepatocellular necrosis (6/15 animals) were seen in the high-dose males, while incidences in the control group were 0/15, 7/15 and 0/15, respectively. Hepatocellular vacuolation was seen in 11/15 high-dose females as compared to an incidence of 5/15 in the control group. At the 2-year sacrifice, megalocytosis was found at an incidence of 0%, 12% and 80% in the males, and 0%, 2% and 16% in the females from the control, low-, and high-dose groups, respectively. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, was observed in 14% and 56% of the low- and high-dose males, as compared to a control incidence of 8%. The incidence of hyperplastic nodules, a localized proliferation of hepatic parenchymal cells, was

slightly increased in the high-dosed males with an incidence of 6% as compared to 0% in the control males.

At the one-year sacrifice, testicular masses were found in 6/50 high-dose and 1/50 low-dose rats, but not in any of the controls. At the termination of the study, there was a significant increase ($p < 0.05$) in the incidence of testicular (Leydig) cell adenomas in the high-dose male rats. The incidence of the Leydig cell tumours in the control, low- and high-dose groups was 0/50 (0%), 2/50 (4%) and 7/50 (14%), respectively.

There was no reported increase in the incidence of pancreatic acinar cell tumours, with incidences of pancreatic acinar hyperplasia in the male rats of 0/33, 2/34, and 1/43 in the control, 30 and 300 ppm groups, respectively. However, the subsequent evaluation of the histopathological slides by an independent pathologist (cited in OECD SIDS) indicated that PFOA produced increased incidences of proliferative acinar cell lesions of the pancreas in the rats at the dietary concentration of 300 ppm. More and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were observed in the study of Biegel et al.[21] compared to the study of Sibinski et al.[20]. The basis for the quantitative differences in the lesions observed is not known but was believed to be due most likely to differences in the diets used in the two laboratories.

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the 2-year sacrifice. The incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. The biological significance of this effect at the time of the initial evaluation was unknown, as there was no evidence of progression to tumours. Recently, however, slides of the ovaries from that study were re-evaluated, with particular emphasis placed on the proliferative lesions of the ovary by Mann and Frame.[26] Using more recently published nomenclature, the ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. No statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined were seen in treated groups compared to controls. There was some evidence of an increase in size of stromal lesions observed at the 300 ppm group; however, adenomas occurred in greater incidences in the control group than in either of the treated groups. Results of this follow-up evaluation indicated that rats sacrificed at the one-year interim sacrifice, as well as rats that died prior to the interim sacrifice were not considered at risk for tumour development in ovaries.

The report also indicated a significant increase ($p < 0.05$) in the incidence of mammary fibroadenomas in both groups of female rats. The incidence of the mammary fibroadenoma was 21% (10/47), 40% (19/47) and 43% (21/49) in the control, 30, and 300 ppm groups, respectively. The increase was also statistically significant when compared to the historical control incidence of 19.0% observed in 1,329 Sprague-Dawley control female rats used in 17 carcinogenicity studies.[27] When the mammary fibroadenoma incidences were compared to the historical control incidence (37%) in 947 female rats in the Haskell Laboratory, there did not appear to be any compound related effect.

The mammary gland findings were re-examined by a Pathology Working Group using diagnostic criteria and nomenclature of the Society of Toxicological Pathologists. The Pathology Working Group (PWG) concluded that there were no statistically significant differences in the incidence of fibroadenoma, adenocarcinoma, total benign neoplasms or total malignant neoplasms of the mammary glands between control and treated animals using Fischer's Exact Test for pair-wise comparison. There was also no significant difference in combined benign and malignant neoplasms between control and treated groups. The main difference between the original reported findings and the PWG results involved findings initially reported as lobular hyperplasia which the PWG classified as fibroadenoma, mostly in the control group. According to the PWG, the incidence of mammary fibroadenoma in the control, low- and high-dose groups were: 32% (16/50), 32% (16/50), and 40% (20/50), respectively.

Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high-dose male and female rats as compared to control values. Clinical chemistry changes included slight (less than 2-fold), but significant increases ($p < 0.05$) in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. Slight (up to about 10%) increases in absolute or relative liver and kidney weights were noted in both high-dose male and female rats at the 1 year interim sacrifice and at the terminal necropsy; however, only the relative liver weight (vs. body weight or brain weight) increases in the high-dose males were statistically significant ($p < 0.05$).

The Committee notes that mammary fibroadenomas and Leydig cell adenomas are considered benign, and that the study is essentially negative for development of malignant cancers.

In a study by Biegel et al. (2002)[21] CD male rats (n=56) were treated in a feed study with 300 ppm (13.6 mg/kg/day) APFO for 2 years. The goal of the study

was to investigate the relationship between peroxisome-proliferating compounds (APFO) and Leydig cell adenomas and pancreatic acinar cell hyperplasia/ adenocarcinoma formation and to test the hypothesis that peroxisome proliferating compounds induce a tumour triad (liver, Leydig cell and pancreatic acinar cells). Two control groups were used (n=80), with one group receiving food *ad libitum*, and one group receiving the same amount of food as the APFO-treated group consumed. Rats were euthanized at interim time points 1, 3, 6, 9, 12, 15, 18 and 21 months for pathological evaluation. At each time point 6 rats/ group were selected for evaluation of cell proliferation and 6 rats/group for evaluation of peroxisome proliferation. For the cell proliferation evaluation the following tissues were collected: testes, epididymides, liver, duodenum, pituitary and all organs with gross lesions. Six days prior to euthanization at each time point, animals designated for cell proliferation evaluation were anesthetized and osmotic pumps containing 20 mg/ml 5-bromo-2'-deoxyuridine (BrdU) were implanted subcutaneously for the measurement of cell proliferation in hepatocytes and Leydig cells. The duodenum was used as positive control. For each cell tissue type 1,000 cells were scored.

For peroxisome proliferation testing the liver and testes were selected. The β-oxidation activity from the liver and Leydig cell peroxisomes was measured at all interim time points. At the end of the 24 month period all surviving rats were sacrificed and organs were examined for gross necropsy and histopathological examination.

Treatment with APFO increased the incidence of hepatocellular adenomas (13% vs. 3% in *ad libitum* controls or 1% in controls pair-fed (both statistically significant, $p < 0.05$)). No hepatocellular carcinomas were observed in the treated group. APFO also increased the incidence of Leydig cell adenomas (11% vs. 0% in *ad libitum* controls and 3% in pair-fed controls (both statistically significant, $p < 0.05$)), and Leydig cell hyperplasia (46% vs. 14% in the *ad libitum* control group (statistically significant, $p < 0.05$) and 33% in the pair-fed controls (not statistically significant)). The incidence of acinar cell hyperplasia was increased in the treated group (39% vs. 18% ($p < 0.05$) in the *ad libitum* control group and 10% ($p < 0.05$) in the pair-fed controls). The incidence of pancreatic acinar cell adenomas was also increased by APFO (9% vs. 0% in the *ad libitum* controls and 1% in the pair-fed control groups (both statistically significant, $p < 0.05$). A single pancreatic gland carcinoma was also observed in one APFO-treated rat.

Treatment with APFO induced a significant increase in relative liver weight at all time points, except at 24 months, which was only significantly increased when compared to the pair-fed controls. Hepatic β-oxidation activity was

significantly elevated at all times when compared to either *ad libitum* or pair-fed controls; however, hepatic cell proliferation was not increased. Absolute testes weights were increased at 24 months. The Leydig cell β-oxidation and cell proliferation were not altered at any sampling time, indicating that the substance did not induce peroxisomes in Leydig cells. Pancreatic acinar cell proliferation was increased at 15, 18, and 21 months when compared to both control groups. Serum estradiol was increased during the first year of the study when compared to both control groups. Serum luteinizing hormone (LH) was significantly elevated at 6 and 18 months and numerically increased at the 9 and 21 months time points. Although not always statistically significant, serum prolactin concentrations were increased at 1, 3, 6, 9 and 12 months time points. Serum follicle-stimulating hormone (FSH) was significantly increased at 6 months time point, while changes in serum testosterone did not show any consistent pattern.

The authors concluded that the obtained data support the hypothesis that APFO, as a peroxisome-proliferating compound, induces the previously described tumour triad. APFO did not induce peroxisomes in Leydig cells, suggesting that the induction of Leydig cell tumours occurs via a different mechanism than the induction of liver tumours. The authors postulated that the Leydig cell tumours are hormonally mediated where the sustained increase in estradiol may play a key role. It has been demonstrated that peroxisome proliferators increase serum estradiol levels via induction of aromatase.[28] Indeed, APFO produced a sustained increase in serum estradiol concentration after 1 month of dietary administration. The authors suggested that estradiol modulates growth factor expression in the testis to produce Leydig cell hyperplasia and neoplasia. The induction of pancreatic acinar cell tumours in rats has been showed to be modified by several factors such as steroid concentration (testosterone and estradiol), growth factors, cholecystokinin (CCK) and diet (fat).[29-31] The authors suggested that APFO may induce pancreatic acinar cell tumours by increasing the fat content in the gut, presumably by enhanced excretion of cholesterol/triglycerides in the liver. The increased fat content in the intestine would increase CCK release into the bloodstream, enhancing pancreatic cell hyperplasia and eventual formation of adenomas.

The Committee notes that the hepatocellular, Leydig cell and pancreatic tumours observed are benign (adenomas).

3.3.2 Studies with monkeys

To understand the potential toxicological response of primates to peroxisome proliferating compounds, Butenhoff and coworkers[22] tested the carcinogenicity

of APFO in Cynomolgus monkeys. Male monkeys were treated with 0 mg/kg bw/day (control group, n=6), 3 mg/kg bw/day (low-dose group, n=4), 10 mg/kg bw/day (mid-dose group, n=6), and 30 mg/kg bw/day, later reduced to 20 (30/20) mg/kg bw/day on Day 22 (high-dose group, n=6). The test substance was given orally (capsules) once a day for 26 weeks. Two monkeys from each of the control and 10 mg/kg bw/day dose groups were observed for 90 days after the last dose to investigate possible recovery of the effects. In addition to observing descriptive toxicity endpoints, the authors aimed at assessing the effect of chronic (26-week) APFO treatment on biological markers associated with the hepatic, pancreatic, and testicular responses seen in the rats with APFO and other peroxisome proliferating compounds. These biological markers included measurement of acyl CoA oxidase activity, replicative DNA synthesis, hormone levels including estradiol and CCK, as well as indications of cholestasis, including bilirubin, alkaline phosphatase, and bile acid determination.

The absolute liver weights were increased ($p < 0.01$) for all groups (35%, 38% and 50 % in the low, mid and high dose, respectively), although relative liver weight increase was statistically significant only in the high dose group. This hepatomegaly, which is often an early sign of peroxisome proliferating carcinogenesis, was not accompanied by any notable histological finding in all dose groups. The authors suggested that the liver weight increase was at least in part due to hepatocellular hypertrophy, as evidenced by decreased hepatic DNA content, which in turn could be explained by mitochondrial proliferation. The latter was demonstrated by a marked increase in succinate dehydrogenase activity. The effects of APFO treatment noted previously in the rat, which are thought to be related to the occurrence of hepatocellular, pancreatic acinar cell and Leydig cell tumours in the rats were not observed in the study. In particular, there was no increase in peroxisome proliferation as measured by palmitoyl CoA oxidase activity. The approximately 2-fold increase in hepatic palmitoyl CoA oxidase activity at the 30/20 mg/kg bw/day dose level was consistent with previous reports for species that are not particularily responsive to peroxisome proliferating compounds. Estradiol was not increased and testosterone was not decreased. No evidence of cholestasis, as evidenced by changes in bile acids, bilirubin, and alkaline phosphatase were observed, and CCK levels did not differ among control and treated groups. Cell proliferation in liver, pancreas or testes, as demonstrated by replicative DNA synthesis, was not different between control and treated groups. The effects on the liver appeared to be reversible, as no APFO-related effects on terminal body weight and organ weights (absolute or relative) were seen in two 10 mg/kg bw/day recovery monkeys.

The Committee notes that in this study the monkeys were exposed for six months only.

3.3.3 *Tumour-promoting studies*

Abdellatif and co-workers studied the modulating action of various peroxisome proliferators, including PFOA, on neoplasia in male Wistar rats.[23-25] The aim was to test the ability of PFOA to act as a positive modulator of hepatocarcinogenesis. The authors used two protocols, a biphasic (initiation - promotion) and a triphasic (initiation - selection - promotion) one. The first one involved initiation by a single intraperitoneal injection of 200 mg/kg bw diethylnitrozamine, followed by 2 weeks recovery and subsequent feeding with a diet containing 0.005% or 0.02% PFOA (no total dose has been reported). Fourteen and twenty five weeks after initiation the rats were sacrificed and their livers used for biochemical and histological analysis. The second protocol used the same initiation, but it was followed by a selection procedure consisting of feeding animals 2-acetylaminofluorene (0.03 w/w) for 2 weeks and in the middle of this treatment an administration of a single necrogenic dose of CCl_4 (2.0 mL/kg bw). Following a recovery period, the rats were fed a diet containing 0.015% PFOA (no total dose has been reported) or 0.05% phenobarbital as a positive control for 25 weeks. The animals were killed and necropsied 7 months after initiation.

In both protocols treatment with PFOA increased the incidence of malignant hepatocellular carcinoma. Twenty five weeks after treatment, the incidence of hepatocellular carcinoma was 14% and 55% (statistically significant, $p < 0.05$) in the animals treated with 0.005% and 0.02% PFOA, respectively (biphasic protocol), and 33% (statistically significant, $p < 0.05$) in the animals treated with 0.015% PFOA (triphasic protocol). The authors also measured the increase in acyl-CoA oxidase activity and catalase activity to study the possible mechanisms involved in tumour promoting activity. The induction of acyl-CoA oxidase activity by PFOA was much stronger than its effect on catalase. Induction figures for acyl-CoA oxidase activity were 7.4 and 14-fold at 14 weeks and 10 and 11-fold at 25 weeks for rats treated with 0.005% and 0.02% PFOA, respectively (biphasic protocol), and 24-fold for rats treated with 0.015% PFOA (triphasic protocol). The induction of catalase activity reached a maximum of 2.3-fold (statistically significant, $p < 0.05$) in animals treated with 0.015 % PFOA (triphasic protocol). The authors concluded that PFOA as a peroxisome proliferator has a positive modulating activity on rat liver carcinogenesis. This is likely to be related to the property of inducing a remarkable increase in

peroxisomal acyl-CoA oxidase activity in comparison to catalase activity, leading to an overproduction of H_2O_2, which subsequently may cause DNA damage.

3.4 Summary of animal carcinogenicity studies

The carcinogenic potential of PFOA has been investigated in two dietary carcinogenicity studies in rats (Biegel et al.[21] and Sibinski[20]). The Sibinski study in rats was essentially negative for any malignant tumour development. The Committee observed that the tumours found were all benign in nature. Under the conditions of the study by Biegel et al., there was evidence that PFOA is carcinogenic, inducing liver tumours, Leydig cell tumours, and pancreatic acinar cell tumours in rats. The liver effects may be explained to a large extent by the mechanism of peroxisome proliferator activated receptor alfa (PPARα) agonism which is well documented for PFOA.[32-34] Leydig cell tumours in rats associated with peroxisome proliferators may result from the hyperplastic effect of sustained increases in serum estradiol due to induction of aromatase[28]. The mechanism for the production of pancreatic acinar cell hyperplasia and tumour formation in rats is less clear, but is thought to be the result of a sustained increase in CCK due to cholestasis.

A six months study in Cynomolgus monkeys[8] did not display the same effects as seen in the rats. The study in monkeys was considered to short to be conclusive with regard to carcinogenic potential of PFOA.

Overall, the Committee concludes that the animal studies show development of benign tumours in rodents, but are negative with respect to malignant tumours.

(The mechanistic aspects will be further discussed in Chapter 5).

Chapter 4

Genotoxicity

4.1 Gene mutation assays

4.1.1 In vitro

Fernandez Freire and co-workers[35] tested PFOA in the Ames test using the plate incorporation method in four different *Salmonella typhimurium* strains (TA98, TA100, TA102 and TA104). The cells were incubated for 48 hours at 37°C with PFOA at 100 and 500 µM with and without metabolic activation (rat S9). The positive controls used per plate were 0.5 µg 4-nitroquinoline-N-oxide (for TA98), 1 µg methyl methanesulfonate (for TA100 and TA102) and 50 µg methyl glyoxal (for TA104). In this test the results were negative in all the *Salmonella* strains used, both with and without metabolic activation. No cytotoxicity was noted.

Griffith and coworkers[36] tested APFO for mutagenic activity in the Ames test with *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 and *Saccharomyces cerevisiae* strain D4. The compound was tested in the absence and in the presence of rat S9. The test substance was tested at a range of 0.1-500 µg per plate, except for the strain TA100, for which concentrations of 100, 500 and 1,000 µg per plate were used. The reason for this were the increased revertants at 500 µg per plate observed in the initial test. The test with

and without metabolic activation of the compound did not reveal mutagenic activity.

Oda and coworkers[17] tested PFOA for its genotoxicity using *Salmonella typhimurium* TA1535/pS K 1002 (hisG46, (rfa, uvr B)) in the umu test. The principle of the umu-test is based on the ability of DNA-damaging agents, most of which are potential carcinogens, to induce the umu operon. PFOA was tested at 0-1,000 µM with and without S9, using DMSO as a solvent. PFOA showed no significant increases in β-galactosidase activity at 0-1,000 µM in the absence of S9 mixture. The results were unchanged by metabolic activation with S9 mixture.

In an NTP program[18], the following strains were used in an Ames test with and without S9: *Salmonella typhimurium* TA98, TA100 and *Escherichia coli* pKM101. DMSO was used as a solvent. However, only a very limited summary of the study results was available from the NTP website* precluding an in-depth interpretation. In the *Salmonella typhimurium* strains PFOA was tested at 0-5,000 µg/plate, and in the *Escherichia coli* strain 0-1000 µg/plate was tested. The standard NTP-protocol with a preincubation method was used with methyl methanesulfonate as a positive control for *Escherichia coli*, sodium azide for TA100, and 2-nitrofluorene for TA98. Negative results were observed in TA100 and pKM101 strains, both with and without metabolic activation, while with T98 strain both positive and negative results were observed in the repeats both with and without metabolic activation. The NTP concluded that the results in *Salmonella typhimurium* were inconclusive.

In addition, two other studies in prokaryotic cells were reported. Since the original reports of these studies could not be obtained directly from publically available literature, as they are owned by an industrial company. The summaries of these studies, as reported in the OECD-SIDS, will be given below.[17]

Ammonium perfluorooctanoate (APFO) was tested twice by Lawlor and coworkers[37,38] for its ability to induce mutations in the *S. typhimurium and E. coli* reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen at one dose level in *S. typhimurium* TA1537 when tested without metabolic activation was not reproducible. APFO did not induce mutation in either *S. typhimurium* or *E. coli* when tested either with or without metabolic activation.

* NTP website: http://ntp-server.niehs.nih.gov/ (accessed October 8, 2013).

Zhao and coworkers[39] tested PFOA for its mutagenicity in mammalian cells. Two human-hamster hybrid cell lines were used, normal human-hamster hybrid A_L cells, and the mitochondria deficient ρ^0 A_L cells. The study was conducted to assay the mitochondria-dependent mutagenesis by reactive oxygen species (ROS) induced by PFOA. The A_L cells were exposed to graded concentrations of PFOA ranging from 1-200 µM for different time points (1, 4, 8 or 16 days). ρ^0 A_L cells were exposed to 100 or 200 µM PFOA for the same time points.

Exposure to PFOA increased the mutation frequencies at CD59 gene loci of A_L but not of ρ^0 A_L cells. The average mutation backgrounds of A_L cells at the CD59 locus used in these experiments were about 100 (74-156) mutants per 10^5 survivors. No distinct mutation inductions were seen in cells treated with 1-200 µM for 1, 4 or 8 days; however, after 16 days exposure to 200 µM the mutation fraction was significantly increased ($p < 0.01$) in the A_L cells. No significant changes in mutation frequency were observed in the ρ^0 A_L cells.

PFOA treatment increased intracellular ROS, NO, and O_2^- production in A_L cells. The ROS level was significantly increased at exposure of 100 µM for 1 day (1.6 times as compared to the untreated control). However, no dose response was seen at the increase of doses and duration of exposure. When treated with 100 µM PFOA for 1 day, NO and O_2^- concentrations were significantly increased ($p < 0.01$) when compared with the respective controls. Similarly, there were no further increases in NO and O_2^- concentrations when treated with 200 µM PFOA or with longer exposure time. Dimethyl sulfoxide (DMSO) was further used as a ROS-inhibitor to further asses the mechanism underlying the PFOA-induced mechanism. It was found that 0.5% DMSO significantly reduced the mutation yield by 200 µM PFOA ($p < 0.05$). When ρ^0 A_L cells were treated with 100 and 200 µM PFOA for 1, 4 or 16 days, no significant increase in the intracellular ROS, O_2^- or NO concentrations were observed compared to the controls. The authors postulated that mitochondria-dependent ROS play an important role in the mutagenicity of PFOA.

The Committee noted that no specific inhibitors (such as rotenone or myxothiazol) of the electron transport chain in the mitochondria were used to actually verify the involvement of mitochondria-dependent ROS.[40,41]

In addition, another study on gene mutations in eukaryotic cells was reported. Since the original of this study could not be obtained directly from publically available literature, as it is owned by an industrial company, the summary of this study, as reported in OECD-SIDS, is given below.

Sadhu[42] reported that APFO did not induce gene mutations when tested with or without metabolic activation in the K-1 line of Chinese hamster ovary (CHO) cells in culture. No further details were reported.

4.1.2 *In vivo*

No in vivo gene mutations studies have been recovered.

4.2 Cytogenetic assays

4.2.1 *In vitro*

The genotoxicity of PFOA was assessed in the micronucleus assay with HepG2 cells by Yao and Zhong.[43] Cells were incubated at 37 ºC for 24 hours and subsequently treated with 50-400 µM PFOA for 24 hours. A total of 3,000 binucleated cells were scored for the evaluation of the frequencies of micronuclei. PFOA induced a dose-dependent increase in the frequency of micronuclei in binucleated HepG2 cells from the concentration of 100 µM ($p < 0.05$ or $p < 0.01$), giving a six-fold increase at the concentration of 400 µM.

In addition, a number of data were reported. Since the original studies could not be obtained directly from publically available literature, as they are owned by industrial companies, the summaries of these studies as reported in OECD-SIDS, will be given below.[17]

APFO did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli[44]; NOTOX[45]). No further details were reported.

Murli and coworkers[46,47] tested APFO twice for its ability to induce chromosomal aberrations in Chinese hamster ovarian (CHO) cells. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy.

4.2.2 *In vivo*

No data were publically available. The original studies could not be obtained directly from publically available literature, as they are owned by industrial

companies. The summaries of these studies, as reported in the OECD-SIDS, are given below.

Murli and coworkers[46,48] tested APFO twice in the in vivo mouse micronucleus assay. APFO did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay. No further details were reported.

4.3 Miscellaneous assays

4.3.1 In vitro

Eriksen and coworkers[49] investigated the ability of PFOA to generate reactive oxygen species (ROS) and to induce oxidative DNA damage in the human hepatoma cell line HepG2. The generation of ROS was measured by using of dichlorofluorescein as a fluorochrome. The DNA damage was tested by using the Comet assay. For the ROS production the cells were exposed for 3 hours at 0.4-2000 µM, for the Comet assay the cells were incubated for 24 hours at 100 or 400 µM. DNA damage was determined in the Comet assay by measuring the formation of DNA strand breaks (SB) and oxidative damage to purines (determined by formamidopyrimidine-DNA-glycosylase (FPG)-sensitive sites).

H_2O_2 was used as positive control for ROS generation in the HepG2 cells at 100, 500 and 1,000 µM. PFOA was found to increase ROS production by 1.52 fold, although not in a clear dose response manner as was found for H_2O_2. PFOA was found not to increase DNA-damage as measured by the Comet assay.

Yao and Zhong[43] also used HepG2 cells in the Comet assay to assess the genotoxic potential of PFOA. In this study, also the intracellular generation of reactive oxygen species (ROS) was determined using the dichlorofluorescein diacetate (DCFH-DA) assay. In addition, oxidative DNA damage in PFOA-treated cells was assessed with the immunocytochemical analysis of 8-OHdG (which is a specific biomarker for ROS-induced DNA damage). The test concentrations were 50-400 µM PFOA in the Comet assay and 100-400 µM PFOA in the measurement of intracellular ROS generation. For ROS determination, the cells were exposed to the test substance for 3 hours. In the Comet assay, the cells were suspended in Dulbecco's minimal essential medium with the test substance and incubated for 1 hour. Only cell suspensions with viabilities > 80T were used for determination of DNA damage.

In the Comet assay, PFOA caused a significant increase ($p < 0.05$) in tail moment at all tested concentrations (50, 100, 200 and 400 µM) in a dose-

dependent manner. The authors found a significant increase ($p < 0.01$) in intracellular ROS at all tested concentrations (100, 200 and 400 µM), giving a four-fold increase at 400 µM. In the experiment with immunocytochemical detection of 8-OHdG, nuclei of PFOA-treated cells presented strong positive staining for 8-OHdG. Following PFOA treatment for 3 hours, the staining intensity increased significantly ($p < 0.01$) at all tested concentrations, giving a ten-fold increase at the concentration of 400 µM.

4.3.2 In vivo

Takagi and coworkers[50] tested the effects of PFOA on oxidative DNA damage in vivo. PFOA was administered to 6 weeks old F-344 male rats in a diet *ad libitum* at 0.02% for two weeks, while a separate group received a single intraperitoneal injection of 100 mg/kg bw PFOA. In the feeding study, rats were killed after 2 weeks, while in the intraperitoneal study five rats at each time point were killed 1, 3, 5 and 8 days after the injection. The formation of 8-hydroxyguanosine (8-OH-dG) adducts by hydroxylation at the C8 position of deoxyguanosine residues in DNA was used as a marker for oxidative DNA-damage by active oxygen radicals. For both exposure routes the level of 8-OH-dG (8-OH-dG/10^5 deoxyguanosine) was significantly increased in the liver. Statistically significant increases were evident at days 3 ($p < 0.05$), 5 ($p < 0.01$) and 8 ($p < 0.05$) post-injection, and after two weeks of oral administration ($p < 0.01$). The hepatomegaly was also evident, with the relative liver weights being significantly increased over the controls ($p < 0.01$) in both cases. No increase of 8-OH-dG levels was observed in kidneys, although relative kidney weights were significantly increased in both groups ($p < 0.01$ at 8 days post-injection and after 2 weeks of oral administration).

4.4 Summary of the genotoxicity studies

The majority of the available Ames tests were negative both with and without metabolic activation (Fernandez Freire and co-workers[35]; Griffith and coworkers[36]; Lawlor and coworkers[37,38] (cited in OECD SIDS)), while one from the NTP study[18] gave equivocal results. PFOA showed no significant increases in β-galactosidase activity al 0-1000 µM in the umu test with *Salmonella typhimurium* TA1535/pS K 1002 (hisG46, (rfa, uvr B)) (Oda and coworkers[17]).

In eukaryotic cells, ammonium perfluorooctanoate did not induce gene mutation when tested with or without metabolic activation in the K-1 line of Chinese

hamster ovary (CHO) cells in culture (Sadhu).[42] In a test in with unconventional cell lines (the human-hamster hybrid A_L cells and the mitochondria deficient ρ0 A_L cells) an increase in mutation fraction at the CD59 locus was seen after 16 days (at 200 μM) incubation. The effects were seen in the A_L cells only, and were considered to be ROS-mediated (Zhao and coworkers).[39] Ammonium perfluorooctanoate did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli[44]; NOTOX[45]), but in two assays with Chinese hamster ovary cells it induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation in one case, and only in the presence of metabolic activation in another case (Murli and coworkers[46,47]). It also gave a statistically significant increase in the micronuclei frequency in a dose-dependent manner in HepG2 cells.[43]

PFOA was also found to increase ROS production in HepG2 cells (Eriksen and coworkers[49]; Yao and Zhong[43]). PFOA was found not to increase DNA-damage as measured by the Comet assay in HepG2 cells in one study (Eriksen and coworkers[49]), but gave a positive result in another study with the same cell line (Yao and Zhong[43]).

The information on in vivo genotoxicity properties of PFOA is limited. The limited available data indicate that PFOA is not clastogenic in vivo (Murli and coworkers).[46,48] Takagi and coworkers[50] demonstrated that PFOA can induce oxidative DNA damage in vivo in a study with rats administered the substance in diet for two weeks.

Taken together, these data do not indicate a direct acting mutagenic potential of PFOA. The effects seen are considered to be indirect through the formation of ROS, a property of peroxisome proliferating agents.

Chapter 5

Mode of action

Several in vitro genotoxicity data are available giving somewhat contradictory results. Overall the results of the available Ames tests were negative. In a test with unconventional cell lines[39] (human-hamster hybrid A_L cells and mitochondria-deficient ρ0 A_L cells) an increase in mutation fraction at the CD59 locus was seen after 16 days (at 200 µM) incubation. The effects were seen in the A_L cells only, and were considered to be ROS-mediated. PFOA was also found to increase ROS production in HepG2 cells.[43,49] In one study PFOA was associated with the clastogenic effects measured as an increase in micronuclei, and damage to DNA (measured in the Comet assay) in HepG2 cells.[43] The substance was also found to induce chromosome aberrations and polyploidy in Chinese hamster ovarian (CHO) cells, either with or without metabolic activation.[46,47]

The information on in vivo genotoxicity properties is limited, and the available data indicate that the in vivo PFOA is not clastogenic.

Taken together these data do not indicate a direct acting mutagenic chemical. The effects seen are considered to be indirect through the formation of ROS, a property that relates to peroxisome proliferating agents.

The carcinogenicity study of APFO in rats of Biegel et al.[21] has shown that PFOA induced liver adenomas, Leydig cell adenomas, and pancreatic acinar cell tumours. The study by Sibinski et al.[20] also showed adenomas in the liver and testes and proliferative acinar lesions of the pancreas. Biegel et al.[21] examined

the temporal relationship between relative liver weights, hepatic β-oxidation, hepatic cell proliferation, and hepatic adenomas in CD rats following PFOA exposure. Relative liver weights and hepatic β-oxidation (a measure for peroxisome proliferation) were increased at all time points tested. Hepatic cell proliferation was numerically increased relative to the pair-fed control at 9, 15, 18, and 21 months. The liver endpoints (weight, β-oxidation, and cell proliferation) were all elevated well before the first occurrence of liver adenomas, which occurred after 12 months of treatment.

The effects in the liver are considered to be mediated to a large extent through peroxisome proliferator activated receptor alfa (PPARα) agonism. It has been well documented that PFOA is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of rodents.[32-34] Liver tumours produced by peroxisome proliferating compounds are considered to be derived from the increased oxidative stress and cell proliferation that accompanies an increase in peroxisomes. Cell proliferation and decreased apoptosis lead to clonal expansion of preneoplastic foci and subsequent tumours. However, in addition, several studies suggest that PFOA hepatotoxicity may be caused in part by PPARα-independent routes (SAB Review, 2006[51]; Klaunig, 2012[52]; Post et al., 2012[2]). The Committee is aware of the ongoing extensive research into various mechanistic aspects of these routes both in in vitro and in transgenic animals. The Committee considers these studies relevant to clarify the mechanisms underlying the development of the (benign) tumours in experimental animals, but observes that these studies did not yet lead to definitive conclusions on the exact mechanism(s) relevant for the tumours observed in the animal studies.

The Leydig cell and pancreatic acinar cell tumours appear to result from mechanisms related to, or secondary to hepatic peroxisome proliferation.[52] Biegel and co-workers[21] suggested that Leydig cell tumours in rats associated with peroxisome proliferators were hormonally mediated and may result from the hyperplastic effect of sustained increases in estradiol due to the induction of aromatase[28].

The mechanism for the production of pancreatic acinar cell hyperplasia and tumour formation by certain peroxisome proliferating compounds in rats is less clear, but is thought to be the result of a sustained increase in cholecystokinin (CCK) in the bloodstream due to cholestasis.[32-34]

The occurrence of the triad of hepatocellular, pancreatic acinar cell, and Leydig cell tumours is observed frequently in animal studies with pharmaceuticals.[53] The triad is related to a large extent to peroxisome proliferation, and is likely to

be species-specific. While rodents are particularly sensitive to this phenomenon, primates, including humans, are predominantly nonresponsive[53] This seems consistent with the results reported by Butenhoff and coworkers[22] for Cynomolgus monkeys. The effects of ammonium perfluorooctanoate (APFO), previously noted in rats, which are thought to be related to the occurrence of hepatocellular, pancreatic acinar cell, and Leydig cell tumours were not observed in the study with monkeys i.e. no increase in peroxisome proliferation, no increase in estradiol , no decrease in testosterone no evidence of cholestasis and no differences in CCK levels among control and treated groups. Only hepatomegaly in the absence of notable histopathologic changes was present in all dose groups. However, in this study the monkeys were exposed to APFO for six months only, and the exposure period was therefore too short to confirm the absence of tumour formation.

With regard to the mechanistic information the Committee concludes that the benign tumour development observed in rodents can be explained for the greater part by peroxisome proliferation. Other mechanisms may be involved as well, especially in the liver, but the data are as yet inconclusive.

Chapter 6

Classification

6.1 Evaluation of data on carcinogenicity and genotoxicity

Six epidemiological studies in three worker cohorts which the Committee considered relevant for relationship between PFOA/APFO exposure with cancer were assessed.[6-11] In addition, two epidemiological studies on cancer in residents in the vicinity of a chemical plant[12,13] and three studies in the general population[14-16] were available.

After review the Committee concludes that the conducted studies are of a varying quality and that several of them suffer from significant weaknesses. Several studies report elevated risks for certain types of cancer. Overall however, there is no cancer type that is consistently elevated in these studies. The cancer type of highest concern according to the Committee is kidney cancer. However, no excess of kidney cancer was observed in the 3M worker cohort, kidney cancer risk in the DuPont worker cohort was in the expected range with a small but statistically significant excess in the highest exposed quartile only, and the multinational cohort mortality study by Consonni et al. reported an overall non-significant excess of kidney cancer mortality lacking a dose-response trend or association with duration of exposure.

The reported results of a relatively substantial number of human longitudinal studies have such a high degree of inconsistency that the Committee considers the human data as insufficient for firm conclusions about whether or not a cancer risk exists from exposure to PFOA in these studies.

Only two suitable rat studies on carcinogenicity were available to the Committee (Sibinski, 1987[20]; Biegel et al., 2001).[21] The study in monkeys was considered too short to be conclusive with regard to carcinogenic potential of PFOA (Butenhoff et al., 2002[22]). The Sibinski study in rats was essentially negative for any malignant tumour development. In the other study in rats (Biegel et al.), PFOA was able to induce a triad of hepatocellular, pancreatic acinar cell, and Leydig cell tumours upon dietary administration. The induction of this triad of tumours is considered to be associated to a large extent with PFOA-induced peroxisome proliferation.[52,53]

With regard tot the Leydig cell tumours the Committee is of the opinion that these benign tumours are species (rodent)-specific and unlikely to have relevance for testicular tumour development in humans. Moreover, rats are quantitatively far more sensitive to the development of Leydig cell tumours than men since Leydig cell LH relasing hormone receptors are unique to rats and also have over 10 times more luteinizing homone receptors than men.[54]

In addition, the Committee is of the opinion that also the benign acinar pancreatic tumours are species (rodent)-specific and have no relevance for pancreatic tumour development in humans.

With regard to the liver the Committee is of the opinion that the tumours observed are benign and for a greater part species (rodent) specific. The data to explain tumour formation by other mechanisms than peroxisome proliferation are inconclusive.

The Committee is of the opinion that the benign tumour development in rodents may be explained for the greater part by peroxisome proliferation. Other mechanisms may be involved as well, especially in the liver, but the data as yet are inconclusive.

Taken together, the Committee concludes that the animal data are insufficient to evaluate the carcinogenicity of PFOA and its salts.

The limited available genotoxicity studies indicate that PFOA is not clastogenic in vivo (Murli and coworkers[46,48]). Takagi and coworkers[50] demonstrated that PFOA can induce oxidative DNA damage in vivo in a study with rats.

The Committee concludes that these data do not indicate a direct acting mutagenic potential of PFOA. The effects seen are considered to be indirect.

6.2 Recommendation for classification

The Committee concludes that the available data on PFOA and its salts are insufficient to evaluate the carcinogenic properties (category 3).*

* According to the classification system of the Health Council (see Annex F).

References

1 Steenland K, Fletcher T, Savitz DA. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). Environ Health Perspect 2010; 118(8): 1100-1108.

2 Post GB, Cohn PD, Cooper KR. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Environ Res 2012; 116: 93-117.

3 ECHA. Ammoniumpentadecafluorooctanoate (APFO): Opinion of the committee for risk assessment on a dossier proposing harmonized classification and labelling at community level. 2011. Internet: http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling/-/substance/305/search/pentadecafluoro/term (accessed October 8, 2013).

4 ECHA. Perfluorooctanoic acid (PFOA): Opinion of the committee for risk assessment on a dossier proposing harmonized classification and labelling at community level. 2011. Internet: http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling/-/substance/813/search/pentadecafluoro/term (accessed October 8, 2013.

5 C-8 Science Panel, l. Status Report: Probable Link Evaluation of Cancer. 2012. Internet: www.c8sciencepanel.org (accessed October 8, 2013).

6 Gilliland FD, Mandel JS. Mortality among employees of a perfluorooctanoic acid production plant. J Occup Med 1993; 35(9): 950-954.

7 Alexander BH. Mortality study of workers employed at the 3M Cottage Grove facility. Division of Environmental and Occupational Health, School of Public Health, University of Minnesota. U.S. Environmental Protection Agency. 2001: Administrative Record 226-1030a018.

8 Lundin JI, Alexander BH, Olsen GW, Church TR. Ammonium perfluorooctanoate production and occupational mortality. Epidemiology 2009; 20(6): 921-928.

9 Leonard RC, Kreckmann KH, Sakr CJ, Symons JM. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Ann Epidemiol 2008; 18(1): 15-22.

10 Steenland K, Woskie S. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am J Epidemiol 2012; 176(10): 909-917.

11 Consonni D, Straif K, Symons JM, Tomenson JA, van Amelsvoort LG, Sleeuwenhoek A et al. Cancer risk among tetrafluoroethylene synthesis and polymerization workers. Am J Epidemiol 2013; 178(3): 350-358.

12 Vieira VM, Hoffman K, Shin HM, Weinberg JM, Webster TF, Fletcher T. Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. Environ Health Perspect 2013; 121(3): 318-323.

13 Barry V, Winquist A, Steenland K. Perfluorooctanoic Acid (PFOA) Exposures and Incident Cancers among Adults Living Near a Chemical Plant. Environ Health Perspect 2013; 121(11-12): 1313-1318.

14 Eriksen KT, Sørensen M, McLaughlin JK, Lipworth L, Tjønenland A, Overvad K et al. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general danish population. J Natl Cancer Inst 2009; 101(8): 605-609.

15 Bonefeld-Jorgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Kruger T et al. Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: a case control study. Environ Health 2011; 10: 88.

16 Hardell E, Karrman A, van BB, Bao J, Carlberg M, Hardell L. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environ Int 2013; 63C: 35-39.

17 oecd sids. SIDS Initial Assessment Report after SIAM 26 (Paris, France, 16-18 April 2008) on Ammonium perfluorooctanoate & Perfluorooctanoic acid. 2008.

18 Kreckmann KH, Sakr CJ, Leonard RC, Dawson BJ. Estimation and validation of biomarker-based exposures for historical ammonium perfluorooctanoate. J Occup Environ Hyg 2009; 6(9): 511-516.

19 Sleeuwenhoek A, Cherrie JW. Exposure assessment of tetrafluoroethylene and ammonium perfluorooctanoate 1951-2002. J Environ Monit 2012; 14(3): 775-781.

20 Sibinski LJ. Final report of a two year oral (diet) toxicity and carcinogenicity study of fluorochemical FC-143 (perfluorooctanane ammonium carboxylate) in rats. 1987: Vol.1-4, 3M Company/RIKER Exp. No.0281CR0012; 8EHQ-1087-0394, October 16, 1987.

21 Biegel LB, Hurtt ME, Frame SR, O'Connor JC, Cook JC. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. Toxicol Sci 2001; 60(1): 44-55.

22 Butenhoff J, Costa G, Elcombe C, Farrar D, Hansen K, Iwai H et al. Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. Toxicol Sci 2002; 69(1): 244-257.

23 Abdellatif AG, Préat V, Vamecq J, Nilsson R, Roberfroid M. Peroxisome proliferation and modulation of rat liver carcinogenesis by 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, perfluorooctanoic acid and nafenopin. Carcinogenesis 1990; 11(11): 1899-1902.

24 Abdellatif AG, Preat V, Taper HS, Roberfroid M. The modulation of rat liver carcinogenesis by perfluorooctanoic acid, a peroxisome proliferator. Toxicol Appl Pharmacol 1991; 111(3): 530-537.

25 Abdellatif AG, Al-tonsy AH, Mahmoud EA, Roberfroid M, Khan MNU. Peroxisomal enzymes and 8-hydroxydeoxyguanosine in rat liver treated with perflurooctanoic acid. Disease Markers 2003; 19(1): 19-25.

26 Mann PC and Frame SR. FC-143: Two year oral toxicity-oncogenicity study in rats: Peer review of ovaries. DuPont Project ID 15261. 2004: June 25, 2004. U.S. Environmental Protection Agency Administrative Record 226.

27 Chandra M, Riley MGI, Johnson DE. Spontaneous neoplasms in aged Sprague-Dawley rats. Arch Toxicol 1992; 66(7): 496-502.

28 Biegel LB, Liu RCM, Hurtt ME, Cook JC. Effects of Ammonium Perfluorooctanoate on Leydig-Cell Function: In Vitro, in Vivo, and ex Vivo Studies. Toxicol Appl Pharmacol 1995; 134: 18-25.

29 Longnecker DS. Early morphologic markers of carcinogenicity in rat pancreas. In: Milman H, Sell S, editors. Application of Biological ?Markers to Carcinogen Testing. New York: Plenum; 1983: 43-60.

30 Longnecker DS. Interface between adaptive and neoplastic growth in the pancreas. Gut 28[Suppl], 253-258. 1987.

31 Longnecker DS, Sumi C. Effects of sex steroid hormones on pancreatic cancer in the rat. Int J Pancreatol 1990; 7[1-3], 159-165.

32 Maloney EK, Waxman DJ. Trans-Activation of PPARalpha and PPARgamma by structuraly diverse environmental chemicals. Toxicol Appl Pharmacol 1999; 161(2): 209-218.

33 Takacs ML, Abbott BD. Activation of mouse and human peroxisome proliferator-activated receptors (alpha, beta/deta, gamma) by perfluorooctanoic acid and perfluorooctanoate sulfonate. Toxicol Sci 2007; 95(1): 108-117.

34 Heuvel JP van den, Thompson JT, Frame SR, Gillies PJ. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison fo human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. Toxicol Sci 2006; 92(2): 476-489.

35 Fernandez Freire P, Perez Martin JM, Herrero O, Peropadre A, de la Pena E, Hazen MJ. In vitro assessment of the cytotoxic and mutagenic potential of perfluorooctanoic acid. Toxicol in Vitro 2008; 22(5): 1228-1233.

36 Oda Y, Nakayama S, Harada KH, Koizumi A. Negative results of umu genotoxicity test of fluorotelomer alcohols and perfluorinated alkyl acids. Env Health Prev Med 2007; 12(5): 217-219.

37 Lawlor TE. Mutagenicity test with T-6342 in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay. 1995: Laboratory Number: 17073-0-409. Corning Hazleton Inc., Vienna, VA. 3M Company. St. Paul, MN. U.S. Environmental Protection Agency Administrative Record 226-0436.

38 Lawlor TE. Mutagenicity test with T-6564 in the Salmonella-Escherichia coli/mammalian-microsome reverse mutation assay with a confirmatory assay. 1996: Corning Hazleton Inc. Final

Report. CHV Study No: 17750-0-409R. September 13, 1996. U.S. Environmental Protection Agency Administrative Record 226-0432.

39 Zhao G, Wang J, Wang X, Chen S, Zhao Y, Gu F et al. Mutagenicity of PFOA in Mammalian Cells: Role of Mitochondria-Dependent Reactive Oxygen Species. Environ Sci Technol 2011; 45[4], 1638-1644.

40 Kuka S, Tatarkova Z, Racay P, Lehotsky J, Dobrota D, Kaplan P. Effect of aging on formation of reactive oxygen species by mitochondria of rat heart. Gen Physiol Biophys 2013; 32(3): 415-420.

41 Zoer B, Cogolludo AL, Perez-Vizcaino F, De Mey JG, Blanco CE, Villamor E. Hypoxia sensing in the fetal chicken femoral artery is mediated by the mitochondrial electron transport chain. Am J Physiol Regul Integr Comp Physiol 2010; 298(4): R1026-R1034.

42 Sadhu D. CHO/HGPRT forward mutation assay - ISO (T6.889.7). 2002: Toxicon Corporation, Bedford, MA. Report No. 01-7019-G1, March 28, 2002. U.S. Environmental Protection Agency Administrative Record 226-1101.

43 Yao X, Zhong L. Genotoxic risk and oxidative DNA damage in HepG2 cells exposed to perfluorooctanoic acid. Mutat Res 2005; 587(1-2): 38-44.

44 Murli H. Mutagenicity test on T-6342 measuring chromosomal aberrations in human whole blood lymphocytes with a confirmatory assay with multiple harvests. 2011: Corning-Hazelton, Inc., Vienna, VA. Study No. 17073-0-449CO, November 1, 1996. U.S. Environmental Protection Agency Administrative Record 226-0433.

45 Notox. Evaluation of the ability of T-7524 to induce chromosome aberrations in cultured peripheral human lymphocytes. 2000: Notox Project Number 292062. Hertogenbosch, The Netherlands.

46 Murli H. Mutagenicity test on T-6342 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. 1996: Corning-Hazelton, Inc. Vienna, VA. Study No. 17073-0-437CO, September 16, 1996. U.S. Environmental Protection Agency Administrative Record 226-0434.

47 Murli H. Mutagenicity test on T-6564 measuring chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with a confirmatory assay with multiple harvests. 1996: Corning Hazleton Inc., Vienna, VA. Study No. 17750-0-437CO, September 16, 1996. U.S. Environmental Protection Agency Administrative Record 226-0431.

48 Murli H. Mutagenicity test on T-6564 in an in vivo mouse micronucleus assay. 1996: Corning Hazleton Inc., Vienna, VA. Study number 17750-0-455, November 1, 1996. U.S. Environmental Protection Agency Administrative Record 226-0430.

49 Eriksen KT, Raaschou-Nielsen O, Sorensen M, Roursgaard M, Loft S, Moller P. Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHxA in human HepG2 cells. Mutat Res 2010; 700(1-2): 39-43.

50 Takagi A, Sai K, Umemura T, Hasegawa R, Kurokawa Y. Short-term exposure to the peroxisome proliferators, perfluorooctanoic acid and perfluorodecanoic acid, causes significant increase of 8-hydroxydeoxyguanosine in liver DNA of rats. Cancer Lett 1991; 57(1): 55-60.

51 USEPA. Science advisory board (SAB) review of EPA's draft risk assessment of potential human health effects associated with PFOA and its salts. 2006. Internet: www.epa.gov/sab/pdf/sab_06_006.pdf (accessed October 9, 2013).

52 Klaunig JE, Hocevar BA, Kamendulis LM. Mode of Action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and Human Relevance. Reprod Toxicol 2012; 33(4): 410-418.

53 Klaunig JE, Babich MA, Baetcke KP, Cook JC, Corton JC, David RM et al. PPARalpha agonist-induced rodent tumors: modes of action and human relevance. Crit Rev Toxicol 2003; 33(6): 655-780.

54 Cook JC, Klinefelter GR, Hardisty JF, Sharpe RM, Foster PM. Rodent Leydig cell tumorigenesis: a review of the physiology, pathology, mechanisms, and relevance to humans. Crit Rev Toxicol 1999; 29(2): 169-261.

55 Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague, The Netherlands: 2010: publication no. A10/07E.

A	Request for advice
B	The Committee
C	The submission letter (in English)
D	Comments on the public review draft
E	IARC Monograph
F	Carcinogenic classification of substances by the Committee

Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in Annex B.

Annex **B**

The Committee

- R.A. Woutersen, *chairman*
  Toxicologic Pathologist, TNO Innovation for Life, Zeist; Professor of Translational Toxicology, Wageningen University and Research Centre, Wageningen
- J. van Benthem
  Genetic Toxicologist, National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  Toxicologist, SHELL International BV, The Hague
- G.J. Mulder
  Emeritus Professor of Toxicology, Leiden University, Leiden
- Ms M.J.M. Nivard
  Molecular Biologist and Genetic Toxicologist, Leiden University Medical Center, Leiden
- G.M.H. Swaen
  Epidemiologist, Dow Chemicals NV, Terneuzen *(until April 1, 2013)*; Exponent, Menlo Park, United States *(from August 15, 2013)*
- E.J.J. van Zoelen
  Professor of Cell Biology, Radboud University Nijmegen, Nijmegen
- G.B. van der Voet, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

The submission letter (in English)

Subject : Submission of the advisory report *Perfluorooctanoic acid and its salts*
Your Reference: DGV/BMO-U-932542
Our reference : U-7970/BV/fs/246-J19
Enclosed : 1
Date : December 18, 2013

Dear Minister,

I hereby submit the advisory report on the effects of occupational exposure to Perfluorooctanoic acid and its salts.

This advisory report is part of an extensive series in which carcinogenic substances are classified in accordance with European Union guidelines. This involves substances to which people can be exposed while pursuing their occupation.

The advisory report was prepared by the Subcommittee on the Classification of Carcinogenic Substances, a permanent subcommittee of the Health Council's Dutch Expert Committee on Occupational Safety (DECOS). The advisory report has been assessed by the Health Council's Standing Committee on Health and the Environment.

I have today sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their consideration.

Yours sincerely,
(signed)
Professor W.A. van Gool,
President

Annex **D**

Comments on the public review draft

A draft of the present report was released in June 2013 for public review. The following organisation and persons have commented on the draft document:

- Dr. T.J. Lentz, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, OH, USA
- Dr. R.A. Billott, Taft Stettinius & Hollister LLP, Cincinnati, OH, USA
- Dr. G.B. Post, New Jersey Department of Environmental Protection, Trenton, NJ, USA.

Annex **E**

IARC Monograph

Perfluorooctanoic acid and its salts have not been evaluated by IARC.

Annex **F**

Carcinogenic classification of substances by the Committee

The Committee expresses its conclusions in the form of standard phrases:

Category	Judgement of the Committee (GR_{GHS})	Comparable with EU Category	
		67/548/EEC before 12/16/2008	EC No 1272/2008 as from 12/16/2008
1A	The compound is known to be carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	1	1A
1B	The compound is presumed to be as carcinogenic to humans. • It acts by a stochastic genotoxic mechanism. • It acts by a non-stochastic genotoxic mechanism. • It acts by a non-genotoxic mechanism. • Its potential genotoxicity has been insufficiently investigated. Therefore, it is unclear whether the compound is genotoxic.	2	1B
2	The compound is suspected to be carcinogenic to man.	3	2
(3)	The available data are insufficient to evaluate the carcinogenic properties of the compound.	not applicable	not applicable
(4)	The compound is probably not carcinogenic to man.	not applicable	not applicable

Source: Health Council of the Netherlands. Guideline to the classification of carcinogenic compounds. The Hague: Health Council of the Netherlands, 2010; publication no. A10/07E.[55]

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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